Commentary

Proteolysis and the Biochemistry of Life-or-Death Decisions

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Apoptosis, the process by which cells commit suicide, is ubiquitous in both normal and diseased tissues, and its mechanism is conserved in animal species from humans at least to nematodes. Apoptotic cells undergo a stereotypical set of changes—shrinkage, cleavage of nuclear DNA, condensation of chromatin, fragmentation of the cytoplasm—that distinguish them from cells dying by other means. In embryogenesis, this process is required to form adult structures (as when digits become separated) and to delete immune cell clones specific for self-antigens, and in adult tissues it removes cells that have suffered damage to their genetic material. Thus, apoptosis helps to establish normal tissues and prevents cell proliferation that could otherwise lead to pathologic conditions such as autoimmunity or cancer.

The recognition that a protein required for apoptosis in the worm, CED-3, is a homologue of the mammalian cysteine proteinase interleukin 1β-converting enzyme (ICE; 1) set the stage for much of the current work on apoptosis. It is now clear that at least five proteinases in the ICE family are expressed in mammalian cells, and that the activity of some or all of these enzymes is required for apoptosis to occur (2). Inappropriate expression of intracellular proteinases including ICE has been shown to drive cells into the apoptotic program. Conversely, normal apoptosis can be prevented by supplying cells with proteinase inhibitors such as CrmA, a viral protein that acts specifically on proteinases of the ICE family (3). On the basis of results such as these, it seems clear that proteolysis is crucially involved in apoptosis, but it is much less clear why this is so. Apoptotic cells do not simply proteolyze themselves out of existence, and at least in the early stages of the process when the cell becomes irreversibly committed to dying, the proteinases induced are extremely limited in their specificity and the number of target proteins is small. Evidently, proteolysis of certain key molecules serves to flip a switch, initiating a series of steps that culminate in the death of the cell and its processing into apoptotic fragments that can be phagocytosed by neighboring cells.

Valuable hints about the nature of this switch come from the work of Casciola-Rosen et al. (4) in this issue of the *Journal of Experimental Medicine*. In previous work (2, 5–10) these and other investigators identified several proteins that are targets of apoptotic proteolysis, and therefore are candidates to be part of the switch. In the current study, they use a specific inhibitor of apopain, a proteinase of the ICE family, and show by several criteria that apopain rather than ICE is responsible for the proteolysis they observe in apoptotic cells. This is consistent with the observation that in mice deficient for ICE, apoptosis still occurs in most cell types. Many of the known proteolytic targets are localized in the nucleus of normal cells: lamins; globular actin; the nuclear mitotic apparatus protein NuMA; the U1-70 kD protein, a component of the RNA splicing machinery; the enzyme poly(ADP-ribose) polymerase (PARP); and the catalytic subunit of the DNA-dependent protein kinase (DNA-PK_{cs}). Of these, U1-70 kD, PARP and DNA-PK_{cs} are believed to be cleaved by apopain (5, 8, 10), and at least the latter two appear to be involved in regulating the cell cycle in response to DNA damage. This function suggests a model for the apoptotic switch in which damage to DNA activates apopain to cleave a set of nuclear proteins that control the progression of cells through the cell cycle or commit it to apoptosis.

DNA Damage and the G1 Checkpoint

Progression of post-mitotic (G1) cells into the DNA synthesis (S) phase of the cell cycle is driven by heterodimeric protein kinases consisting of cyclins and cyclin-dependent kinases (cdk). In late G1, for example, cyclin E pairs with cdk2 to form an active kinase that is required to initiate DNA replication (reviewed in reference 11). When normal cells are exposed to ionizing radiation or other treatments that lead to DNA strand breakage, this progression does not take place. Rather, the inhibitory subunit p21 forms a complex with G1 cyclin-cdk heterodimers, causing the cell to remain in G1 (12). Because this blockade of the cell cycle is typically transient and is induced by damage to the genome, it is described as the G1 DNA damage checkpoint, one of several known control points in the cell cycle. A key regulator of the G1 checkpoint, as well as of DNA repair and apoptosis, is the tumor suppressor protein p53 (Fig. 1).

p53 is a transcription factor that is induced in cells with DNA damaged by ionizing radiation or other treatments (13, 14). p53 initiates the synthesis of p21 and other nuclear proteins, including the growth arrest and DNA damage-induced protein Gadd45, which binds to the proliferating cell nuclear antigen (PCNA). Gadd45 appears to potentiate DNA repair in nuclear extracts (reference 15 but also see reference 16 for some contradictory data). p21 and Gadd45 have distinct roles in the G1 checkpoint. As noted, p21 binds

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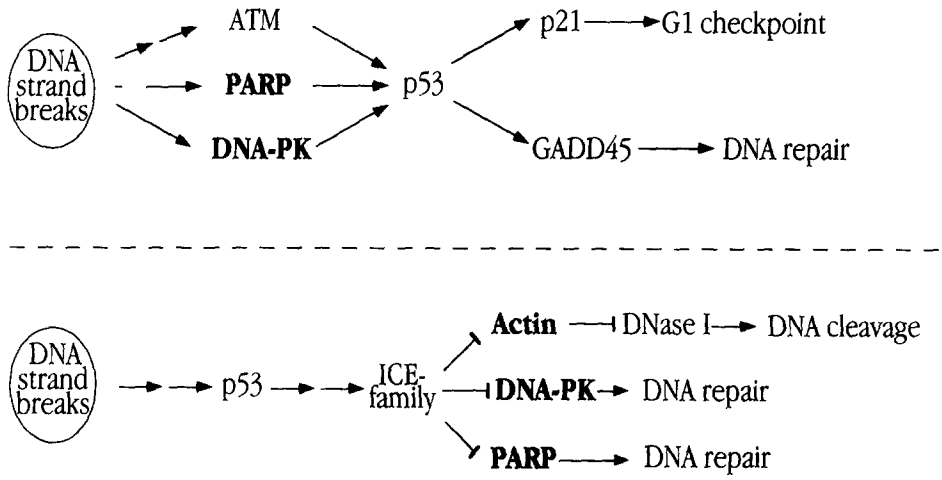

Figure 1. Proposed functions of target substrates (*bold*) of ICE family proteinases in regulating the G1 checkpoint and apoptosis in response to DNA damage. p53 is central to G1 arrest. The upper panels outline the steps leading to cell cycle arrest and DNA repair after damage. p53 may be induced by at least three distinct pathways. The activities of the nuclear enzymes PARP and DNA-PK are induced directly by DNA strand breaks, and contribute to p53 activation. ATM, a major regulator of p53, may induce p53 by an indirect mechanism. p53-dependent cycle checkpoint control is then mediated through induction of transcription of p21, which inhibits the cyclin-cdk complex, and Gadd 45, which binds to PCNA. p53 activation is also central to induction of apoptosis (*bottom*). In this case, p53 induction by DNA damage leads to activation of the ICE family proteinases and, hence, the degradation of the target proteins globular actin, DNA-PK and PARP, whereas degradation of nuclear actin may free DNase I to initiate DNA cleavage. Proteolysis of PARP and DNA-PK_{CS} (and of a number of other potential substrates including ATM, Ref-1 and FRAP) may compromise the DNA repair and cell cycle delay functions shown in the upper panel and could lead to efficient execution of the death program.

to and directly inhibits the S-phase-promoting activity of the cyclin-cdk complex. In addition, p21 forms a complex with PCNA that inhibits DNA polymerase activity, thus directly opposing the process of DNA replication (13). Cells lacking p53 can, by means of uncharacterized pathways, still induce Gadd45 in response to DNA damage, but they proceed into S phase without activating a G1 checkpoint.

Mechanisms of p53 Activation

The induction of p53 by DNA strand breaks is complex and not fully understood. Several mechanisms are involved, but it is not yet clear whether they represent steps on a single pathway or multiple independent pathways, all leading to increased p53 expression or activation. One major regulator of p53 function is the ATM protein, which is deficient in patients suffering from the recessive neurological disorder ataxia-telangiectasia (A-T; reviewed in reference 17). ATM appears from its sequence to be a kinase, and its activity is required for the G1 checkpoint, but it is not yet clear what its substrates are or if they interact with known inducers of S phase. A-T patients' lymphocytes lack the normal G1 checkpoint control. After irradiation, these cells proceed into S phase without repairing chromosomal breaks, and subsequently die by apoptosis (17). This heightened apoptotic response is attributed to misregulation of p53, as described below, and is likely to underlie the nervous system degeneration seen in these patients.

Like ATM, PARP and DNA-PK are believed to contribute to p53 induction. PARP is a modular protein comprising an NH_2-terminal DNA binding domain, an auto-modification domain and a COOH-terminal catalytic domain. PARP binds specifically to sites of DNA damage and becomes activated to transfer ADP-ribose onto various nuclear proteins, including PARP itself (18). The role of this protein modification is uncertain despite considerable interest and speculation, and the mystery was only deepened when PARP-deficient mice were found to be normal in DNA repair and radiosensitivity (19). However, in cell culture experiments with cells either genetically deficient for PARP or depleted of NAD, the substrate of the enzyme, it appears that PARP activity is required for p53 synthesis induced by DNA strand breaks (20). Why these results are not reflected in the phenotype of the mutant animal is a question that will certainly stimulate more research, but it seems likely that cells possess several paths to p53 induction.

DNA-PK is another enzyme activated by DNA breaks (21). The DNA-PK holoenzyme consists of a 350-kD catalytic subunit associated with the proteins Ku70 and Ku80. The Ku proteins bind specifically to DNA lesions and recruit the DNA-PK_{cs} to the site of damage, leading to its activation. Again, although numerous substrates of this enzyme have been identified, their role in downstream events is uncertain. One of these substrates is p53, and it has been suggested that phosphorylation of p53 causes this normally short-lived protein to be stabilized. Whatever the mechanism, DNA-PK seems to be important in the repair of DNA breaks. A defect in the DNA-PK_{cs} is responsible for severe combined immunodeficiency (SCID) in mice. SCID animals are radiosensitive and lack functional immune cells owing to a defect in DNA recombination (22, 23). Because maturation of B and T lymphocytes requires DNA breakage and rejoining in the immunoglobulin and T cell receptor loci before the cells can replicate their DNA, it may be that this process normally activates a G1 checkpoint, just as artificially induced DNA damage does. Absent DNA-PK activity, SCID cells fail to rejoin broken DNA ends, although they have been found to induce G1 arrest after irradiation,

suggesting that p53 can be induced by mechanisms independent of DNA-PK.

The Decision to Stop or Die

DNA strand breaks cause cells to arrest before initiating S phase, but it does not follow that they will remain in G1 indefinitely as repairs proceed. Indeed, depending on experimental conditions, cells may not accumulate in G1 at all, but rather apoptose rapidly and quantitatively. Surprisingly, p53, the modulator that is central to G1 arrest after DNA breaks, is also required to induce apoptosis under the same conditions. In normal cells, drug- or radiation-induced DNA damage causes ICE family proteinases to be activated, committing cells to apoptosis. In cells lacking p53, both the apoptotic response and the G1 checkpoint response to DNA damage are compromised.

If p53 induction initiates both the G1 checkpoint and the apoptotic program, how does a cell determine which path to take? The answer emerging to this critical question is that it opts to do both. Guillouf et al. (24) recently reported that by transfecting p53-deficient cells with a temperature-sensitive p53, they can provoke apoptosis by shifting the culture to the permissive temperature, without radiation or drugs to damage DNA. If, however, they simultaneously transfect cells with cDNA encoding Bcl-2, an inhibitor of ICE activation, cells arrest in G1 and only slowly initiate apoptosis. In both transfected populations, downstream genes regulated by p53 are expressed in a similar manner, including the G1 arrest genes *p21* and *Gadd45* and the apoptotic regulators *Bax* and the endogenous *Bcl-2* (24). These results indicate that p53 activation initiates both pathways, and that which one predominates may reflect the level of anti-apoptotic protection the cell enjoys, in this case from the overexpression of Bcl-2. Others have shown that the presence of cytokines such as insulin-like growth factor-2 can maintain cells in G1 arrest by promoting expression of p21. If this survival factor is depleted at the time of irradiation, cells apoptose rather than remaining in G1 (25). Together, these data show that at least the early events initiating the two pathways are shared and that subtle factors such as cell type and culture conditions and perhaps the nature and extent of DNA damage can force the outcome in either direction. The ATM protein, which has been shown to induce checkpoints at G1 and elsewhere in the cell cycle, appears to inhibit p53-dependent apoptosis (17, 26) but a direct effect of ATM on ICE family proteinases has not been established.

Proteolysis in Apoptosis

Of the known proteolytic substrates cleaved in apoptosis, several would appear to be structural rather than regulatory molecules. The nuclear lamins provide a scaffold underlying the nuclear envelope, and it may be necessary to degrade these proteins to release bound transcription complexes and break the cell down into apoptotic fragments. In addition, the nucleus is a highly organized structure. Disrupting this structure by degrading the lamins may make the nuclear DNA more accessible to cleavage by nucleases, thus promoting internucleosomal DNA cleavage and chromatin condensation, two hallmarks of apoptotic death. The nuclear protein NuMA is phosphorylated and cleaved very early in response to DNA damage in thymocytes, and it has been suggested that these events set the stage for later events such as lamin proteolysis (7, 9). Actin has been found to be cleaved by ICE and possibly other related proteinases (6), and the degradation of such an important cytoskeletal protein would obviously compromise cellular structure and could facilitate cell fragmentation. However, it is not clear that filamentous actin is degraded by ICE. Kayalar and colleagues (6) have proposed a more subtle role for actin proteolysis in apoptosis: activation of DNase I to cleave nuclear DNA. They find that treating nuclear extracts with ICE frees latent DNase I from a catalytically inactive complex with globular actin. This observation could provide a satisfying explanation for several otherwise puzzling observations, namely, the presence of actin in the nucleus, the high affinity binding of actin and DNase I, and the role of actin as a proteolytic target in apoptosis.

Cleavage of PARP and DNA-PK_{cs} may, as Casciola-Rosen et al. suggest (4), serve to cripple the DNA repair mechanisms that operate during G1 arrest. As described above (also see Fig. 1), these proteins act upstream of p53 to induce the G1 checkpoint and promote DNA repair. Therefore, cleaving these molecules could repress p53 and inhibit repair of the DNA damage accompanying apoptotic cell death. Because p53 is required to induce apoptosis in cells with DNA strand breaks, this model predicts that the activation of p53 in apoptotic cells is transient, being limited by the destruction of PARP and DNA-PK_{cs}. p53 activation at the G1 checkpoint, on the other hand, is not subject to such a negative feedback loop and may be maintained as long as DNA damage persists. It is interesting to note that, like PARP and DNA-PK_{cs}, ATM (27) has predicted sites for cleavage by apopain, including one pentapeptide sequence in its putative catalytic domain that closely matches the PARP cleavage site. If ATM, too, should prove to be a target of apopain in apoptotic cells, this would support the idea that p53-dependent repair mechanisms need to be inactivated for apoptosis to proceed efficiently (Fig. 1). Several other proteins involved in DNA repair or regulation of G1 arrest are potential substrates for apopain, including the apurinic-apyrimidinic endonuclease Ref-1 (28, 29), uracil DNA glycosylase, and the PI3-kinase homologue FRAP (see reference 4).

ICE and its relatives are highly specific endoproteinases, and it should be noted that cleavage by such an enzyme need not destroy or inactivate a substrate molecule. It is known, for example, that ICE can activate apopain by proteolysis and apopain appears to be directly responsible for cleaving some or all of the downstream target molecules. Similarly, the cleaved substrates of apopain may exhibit activities not found in the uncleaved molecules. In the case of DNA-PK_{cs}, cleavage by apopain removes the catalytic domain of the molecule from the region that interacts with Ku proteins. This catalytic domain retains basal catalytic activity (4) and because it is no longer localized to sites of DNA damage, it

may have access to a different set of substrates than does the DNA-PK holoenzyme. Similarly, PARP cleavage does not destroy the COOH-terminal catalytic portion of the molecule but separates it from the NH_2-terminal domain that binds DNA. Surprisingly, this NH_2-terminal domain may be biologically active independent of the catalytic portion of the molecule and also independent of its ability to bind DNA. Schreiber et al. (30) found that, compared with parental cells, cells expressing the NH_2-terminal domain of PARP dramatically increased the rate of cell doubling in response to DNA damage, suggesting that the transgene compromises some cell cycle checkpoint. Cells accumulated at some point after S phase and underwent apoptosis and DNA recombination at an elevated rate. These same effects were observed with a mutant NH_2-terminal domain lacking a zinc-finger required for DNA binding (30). Evidently, this portion of the protein can interact with other molecules in addition to DNA, with profound effects on cell cycle control. A crucial question is whether the NH_2-terminal fragment released from PARP by apopain in vivo, which is smaller than the fragment engineered by Schreiber et al., exerts similar effects.

Although the cleavage of ICE family targets during apoptosis facilitates cell death, the process is not completely benign from the standpoint of the organism. A subset of infrequently targeted autoantigens in systemic lupus erythematosus are cryptic nuclear determinants generated by the action of apopain on such substrates as the U1-70 kD protein and DNA-PK_{cs} (10). How this subset of epitopes is targeted for an autoimmune response is not known (31). However, the observation of clustered autoantigens suggests the active participation of cells that have activated apopain-dependent proteolysis but have not (or not yet) undergone apoptosis.

One unresolved issue is at what point apoptosis becomes irreversible after the activation of the ICE family proteinases. Clearly, the induction of ICE and apopain do not necessarily trigger cell death. These proteinases have normal physiological roles in the activation of interleukin 1β and of the sterol regulatory element binding protein transcription factors (32, 33). It is not known whether other targets of these proteinases are cleaved under these conditions, but there is reason to suspect that even low levels of proteolysis could have dramatic effects on p53 function. Cells from A-T heterozygotes have markedly reduced induction of p53 compared to normal cells, and these patients are at increased risk of breast and ovarian cancer (34). These findings suggest that p53 is exquisitely sensitive to ATM expression levels. If the induction of ICE family proteinases under physiological conditions can compromise DNA repair responses or generate autoantigens without inducing apoptosis, the results could be of far greater consequence to the organism than the death of the affected cell.

Toward a Biochemistry of Life and Death

The twin fields of apoptosis and cell cycle research are only now beginning to yield detailed biochemical data. With the identification of the ICE family proteinases, their cleavage specificities and some of their substrates, one can now formulate testable predictions about the physiological role of proteolysis in apoptosis. For example, if the Casciola-Rosen model (4) is correct, namely, that cleavage of DNA repair molecules allows cells to apoptose efficiently, it would be expected that cleavage-resistant repair molecules would extend the time needed for apoptosis to be completed. Fortunately, animals lacking the various substrate proteins now exist, either as spontaneous mutants (the SCID mouse, lacking DNA-PK_{cs}) or by gene targeting (the PARP-deficient mouse). Mutant versions of the proteins that lack the apopain cleavage site can now be designed and can be expressed in cultured cells or whole animals, alone or in combination, in the presence or absence of the wild-type protein. Such experiments should provide a far more satisfying mechanistic understanding of the way that life-or-death decisions are made at the cellular level.

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References

1. Yuan, J., S. Shaham, S. Ledoux, H.M. Ellis, and H.R. Horvitz. 1993. The *C. elegans* cell death gene *ced-3* encodes a protein similar to mammalian interleukin-1β-converting enzyme. *Cell.* 75:641–652.
2. Martin, S.J., and D.R. Green. 1995. Protease activation during apoptosis: Death by a thousand cuts? *Cell.* 82:349–352.
3. Miura, M., R. Rotello, E. A. Hartweig, H. Zhu, and J. Yuan. 1993. Induction of apoptosis in fibroblasts by interleukin-1β-

converting enzyme, a mammalian homolog of the *C. elegans* cell death gene *ced-3*. *Cell.* 75:653–660.
4. Casciola-Rosen, L., D.W. Nicholson, T. Chong, K.R. Rowan, N.A. Thornberry, D.K. Miller, and A. Rosen. 1996. Apopain/CPP32β cleaves proteins that are essential for cellular repair: a fundamental principle of apoptotic death. *J. Exp. Med.* 183:1957–1964.
5. Casciola-Rosen, L., D.K. Miller, G.J. Anhalt, and A. Rosen. 1994. Specific cleavage of the 70-kDa protein component of the U1 small nuclear ribonucleoprotein is a characteristic biochemical feature of apoptotic cell death. *J. Biol. Chem.* 269: 30757–30760.
6. Kayalar, C., T. Ord, M. Pia Testa, L.-T. Zhong, and D.E. Bredesen. 1996. Cleavage of actin by interleukin 1β-converting enzyme to reverse DNase I inhibition. *Proc. Natl. Acad. Sci. USA.* 93:2234–2238.
7. Weaver, V.M., B. Lach, P.R. Walker, and M. Sikorska. 1993. Role of proteolysis in apoptosis: Involvement of serine proteases in internucleosomal DNA fragmentation in immature thymocytes. *Biochem. Cell Biol.* 71:488–500.
8. Tewari, M., L.T. Quan, K. O'Rourke, S. Desnoyers, Z. Zeng, D.R. Beidler, G.G. Poirer, G.S. Salvesen, and V.M. Dixit. 1995. Yama/Cpp32b, a mammalian homolog of ced-3, is a CrmA-inhibitable protease that cleaves the death substrate poly(ADP-ribose) polymerase. *Cell.* 81:801–809.
9. Weaver, V.M., C.E Carson, P.R. Walker, N. Chaly, B. Lach, Y. Raymond, D.L. Brown, and M. Sikorska. 1996. Degradation of nuclear matrix and DNA cleavage in apoptotic thymocytes. *J. Cell Sci.* 109:45–56.
10. Casciola-Rosen, L.A., G.J. Anhalt, and A. Rosen. 1995. DNA-dependent protein kinase is one of a subset of autoantigens specifically cleaved early during apoptosis. *J. Exp. Med.* 182:1625–1634.
11. Cordon-Cardo, C. 1995. Mutations of cell cycle regulators. Biological and clinical implications for human neoplasia. *Am. J. Pathol.* 147:545–560.
12. Evan, G.I., L. Brown, M. Whyte, and E. Harrington. 1995. Apoptosis and the cell cycle. *Curr. Opin. Cell Biol.* 7:825–834.
13. Sanchez, Y., and S.J. Elledge. 1995. Stopped for repairs. *Bioessays.* 17:545–548.
14. Elledge, R.M., and W.-H. Lee. 1995. Life and death by p53. *Bioessays.* 17:923–930.
15. Smith, M.L., I.-T. Chen, Q. Zhan, I. Bae, C.-Y. Chen, T.M. Gilmer, M.B. Kastan, P.M. O'Connor, and A.J. Fornace, Jr. 1994. Interaction of the p53-regulated protein Gadd45 with proliferating cell nuclear antigen. *Science (Wash. DC).* 266:1376–1380.
16. Karantsev, A., A. Sancar, J.M. Kearsey, M.K. Shivji, P.A. Hall, and R.D. Wood. 1995. Technical comments: does the p53 up-regulated Gadd45 protein have a role in excision repair? *Science (Wash. DC).* 270:1003–1006.
17. Meyn, M.S. 1995. Ataxia-telangiectasia and cellular responses to DNA damage. *Cancer Res.* 55:5991–6001.
18. de Murcia, G., V. Schreiber, M. Molinete, B. Saulier, O. Poch, M. Masson, C. Niedergang, and J. Menissier de Murcia. 1994. Structure and function of poly(ADP-ribose) polymerase. *Mol. Cell. Biochem.* 138:15–24.
19. Wang, Z.Q., B. Auer, L. Stingl, H. Berghammer, D. Haidacher, M. Schweiger, and E.F. Wagner. 1995. Mice lacking ADPRT and poly(ADP-ribosyl)ation develop normally but are susceptible to skin disease. *Genes & Dev.* 9:509–520.
20. Whitacre, C.M., H. Hashimoto, M.L. Tsai, S. Chatterjee, S.J. Berger, and N.A. Berger. 1995. Involvement of NAD-poly(ADP-ribose) metabolism in p53 regulation and its consequences. *Cancer Res.* 55:3697–3701.
21. Anderson, C.W. 1993. DNA damage and the DNA-activated protein kinase. *Trends Biochem. Sci.* 18:433–437.
22. Kirchgessner, C.U., C.K. Patil, J.W. Evans, C.A. Cuomo, L.M. Fried, T. Carter, M.A. Oettinger, and J.M. Brown. 1995. DNA-dependent kinase (p350) as a candidate gene for the murine SCID defect. *Science (Wash. DC).* 267:1178–1183.
23. Lees-Miller, S.P., R. Godbout, D.W. Chan, M. Weinfeld, R.S. Day, III, G.M. Barron, and J. Allalunis-Turner. 1995. Absence of p350 subunit of DNA-activated protein kinase from a radiosensitive human cell line. *Science (Wash. DC).* 267:1183–1185.
24. Guillouf, C., X. Grana, M. Selvakumaran, A. DeLuca, A. Giordano, B. Hoffman, and D.A. Liebermann. 1995. Dissection of the genetic programs of p53-mediated G1 growth arrest and apoptosis: blocking p53-induced apoptosis unmasks G1 arrest. *Blood.* 85:2691–2698.
25. Canman, C.E., T.M. Gilmer, S.B. Coutts, and M.B. Kastan. 1995. Growth factor modulation of p53-mediated growth arrest versus apoptosis. *Genes & Dev.* 9:600–611.
26. Enoch, T., and C. Norbury. 1995. Cellular responses to DNA damage: cell cycle checkpoints, apoptosis and the roles of p53 and ATM. *Trends Biochem. Sci.* 20:426–430.
27. Savitsky, K., A. Bar-Shira, S. Gilad, G. Rotman, Y. Ziv, L. Vanagaite, D.A. Tagle, S. Smith, T. Uziel, and S. Sfez. 1995. A single ataxia-telangiectasia gene with a product similar to PI-3 kinase. *Science (Wash. DC).* 268:1749–1753.
28. Xanthoudakis, S., G.G. Miao, and T. Curran. 1994. The redox and DNA-repair activities of Ref-1 are encoded by nonoverlapping domains. *Proc. Natl. Acad. Sci. USA.* 91:23–27.
29. Robson, C.N., and I.D. Hickson. 1991. Isolation of cDNA clones encoding a human apurinic/apyrimidinic endonuclease that corrects DNA repair and mutagenesis defects in *E. coli* xth (exonuclease III) mutants. *Nucl. Acids Res.* 19:5519–5523.
30. Schreiber, V., D. Hunting, C. Trucco, B. Gowans, D. Grunwald, G. de Murcia, and J. Menissier de Murcia. 1995. A dominant-negative mutant of human poly(ADP-ribose) polymerase affects cell recovery, apoptosis, and sister chromatid exchange following DNA damage. *Proc. Natl. Acad. Sci. USA.* 92:4753–4757.
31. Lanzavecchia, A. 1995. How can cryptic epitopes trigger autoimmunity? *J. Exp. Med.* 181:1945–1948.
32. Wang, X., R. Sato, M.S. Brown, X. Hua, and J.L. Goldstein. 1994. SREBP-1, a membrane-bound transcription factor released by sterol-regulated proteolysis. *Cell.* 77:53–62.
33. Wang, X., J.-t. Pai, E.A. Wiedenfeld, J.C. Medina, C.A. Slaughter, J.L. Goldstein, and M.S. Brown. 1995. Purification of an interleukin-1β converting enzyme-related cysteine protease that cleaves sterol regulatory element-binding proteins between the leucine zipper and transmembrane domains. *J. Biol. Chem.* 270:18044–18050.
34. Birrell, G.W., and J.R. Ramsey. 1995. Induction of p53 protein by gamma radiation in lymphocyte lines from breast cancer and ataxia telangiectasia patients. *Br. J. Cancer.* 72:1096–1101.